INFECTIVE PROPERTIES OF ROUS VIRUS RNA AND OF TOTAL RNA FROM TUMORS INDUCED BY THIS VIRUS

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Preparations of total RNA obtained from Rous fowl sarcoma possessed infective activity which was not inhibited by ribonuclease, desoxyribonuclease, or specific antiserum against the virus. RNA isolated from Rous virus concentrated by ultra centrifugation was biologically inactive.

Purified nucleic acids of many small viruses of animals possess infective properties. Meanwhile, attempts to isolate infective nucleic acid from viruses with more complex organization are frequently unsuccessful. In some cases, however, infective nucleic acid has been obtained from such viruses, notably from influenza [2, 3, 6, 11-13] and Sendai [10] viruses.

In the investigation described below, infective activity of Rous virus RNA and also of total RNA from a tumor induced by this virus was studied.

EXPERIMENTAL METHOD

Rous sarcoma virus of strains Schmidt-Ruppin and D-5 was used as the culture fluid from an infected culture of chick embryonic cells. The virus was concentrated by centrifugation at 30,000 g (at the upper mark on the 40 rotor of a Spinco L-50 ultracentrifuge). The virus content in the resulting material, when titrated on the basis of transformation plaques in a culture of chick embryonic cells, was $10^{6.8}$-$10^{7.5}$ PFU/ml.

RNA was isolated directly from concentrated Rous virus or from tissues of a sarcoma induced by this virus in a fowl. For this purpose the phenol-detergent method [1, 5] was used, for it yields an RNA in the most native state and of the highest degree of purity. RNA isolated from the tumor by this method is total. Since this procedure includes treatment of the RNA preparations with desoxyribonuclease, when used on such objects as brain, spleen, liver, and connective tissue, it enabled all DNA to be removed from the RNA preparations. On the other hand, when RNA was isolated from Rous sarcoma, even after two 30-min treatments with desoxyribonuclease, not all DNA could be removed from the preparations.

The infective activity of the RNA was studied by adding it in a dose of 10-50 μg/ml to a culture of chick embryonic cells and then testing the oncogenic activity of the experimental cultures on chickens. In addition, RNA preparations were tested immediately after they were obtained for their oncogenic activity on chickens, newborn Syrian hamsters, and BALB/c mice by subcutaneous injection of the isolated RNA in a dose of 0.2 mg. The virus and induced RNA were identified by the neutralization test in a culture of chick embryonic cells, using immune strain-specific rabbit sera.

In control experiments RNA was incubated with ribonuclease, taken in a concentration of 30 μg/ml for 30 min at 37°. To exclude the presence of intact virus particles in the test material, all RNA prepara-

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TABLE 1. Detection of Infective Activity of Total RNA Isolated from Rous Fowl Sarcoma

Strain of virus used to induce tumor	Incubation of RNA with ribonuclease before addition to culture	Test for presence of infective virus in culture		Ability of virus to be neutralized by strain-specific antiserum
		transformation plaques in culture	oncogenic activity on chickens	
Schmidt-Ruppin	+	13/14	6/9	+
	−	15/15	9/10	+
D-5	+	10/16	6/10	+
	−	8/15	5/10	+

Note. Aggregated data of two experiments given in table. Numerator shows number of cultures with transformation plaques or of chickens with tumor; denominator gives total number of cultures or chickens used in experiments.

tions were added mixed (1:1) with a 1:40 dilution of heated (1 h at 60°) strain-specific antiserum, capable of neutralizing in this dilution about 10^3 PFU/ml of native Rous sarcoma virus.

RESULTS

RNA preparations obtained from concentrated Rous virus possessed no infective properties. Total RNA isolated from tumor tissue likewise possessed no oncogenic activity when injected into chickens, hamsters, and mice. Meanwhile, after treatment of a monolayer of chick embryonic cells with total RNA from Rous sarcoma in culture, transformation plaques typical of Rous sarcoma virus appeared after 8-12 days. The culture fluid and cells of these cultures contained normal infective virus particles, as shown by the possibility of inducing tumors in chickens by this material, of subculturing the infective material, and by the ability of a strain-specific antiserum to neutralize the newly synthesized virus (Table 1).

The fact was noted that preincubation of the test RNA with ribonuclease before its addition to the culture did not cause the preparation to lose its infective activity. Since RNA of Rous virus is sensitive to the destructive action of this enzyme [14], these results were evidence that in these experiments it was not the activity of RNA of Rous virus itself that was revealed. The effect observed likewise could not be associated with the presence of intact virus particles in the test material. This was ruled out, first, by the fact that the preparation was injected together with antiserum against the virus and, second, by the technique used to prepare RNA from the cell homogenate, during which the homogenate was treated ten times with phenol and subsequently the RNA was reprecipitated twice or three times with ethyl alcohol. Judging from data in the literature [8], this method of isolation of nucleic acid reliably frees the resulting material from intact virus particles.

Most probably the biological activity of the tested preparations was due to their content of a replicative form of RNA, which is known to possess infective properties and, at the same time, to be resistant to the hydrolytic action of ribonuclease [7]. However, no report could be found in the literature regarding the formation of a replicative form of RNA in cells infected with Rous virus.

Since reproduction of Rous virus evidently must involve the synthesis of a new DNA template in the cell, and be directly dependent on its functions [16], the biological activity of the tested preparations could be due to the presence of this DNA in them (traces of DNA, as was pointed out in the section "Experimental Method," were always present in the preparations despite their repeated treatment with desoxyribonuclease).

It is also very possible that the observed effect could be due to the presence of RNA-DNA hybrids in the tested preparations, for these also are resistant to the action of ribonuclease [15].

The experiments thus demonstrated the infective activity of total RNA preparations obtained from Rous fowl sarcoma. Possible causes of the infectivity of the preparations could be that they contain a replicative form of RNA, a virus DNA template which was not removed during treatment of the cell homogenate, or biologically active RNA-DNA hybrids. The infective activity of RNA from Rous virus itself could thus be detected.

According to one report in the literature [4], an attempt to obtain infective RNA from Rous sarcoma was unsuccessful: this worker isolated RNA from a cell-free tumor homogenate by treating it with acid

phenol without detergent, and he then tested the resulting RNA on animals. After injection of RNA isolated by this method into chick embryos, some of the embryos died [9]. This was associated with infective activity of the "tumor" RNA, although no specific lesions, in the form of plaques on the chorioallantoic membrane, were found in these investigations. When cultures of chick fibroblasts were treated with RNA isolated from Rous sarcoma with neutral hot phenol, sometimes changes were observed which were similar to those caused by the virus [17]. However, no active Rous virus could be discovered in these cultures.

The positive results of isolation of infective RNA from Rous sarcoma in the present experiments can perhaps be explained by the fact that whole tumor homogenate was used for preparation of the RNA and was treated by alkalified phenol with detergent. By this method, preparations containing all types of cell nucleic acids could be obtained. The final condition for successful detection of infective activity of the preparations was that they were tested in tissue cultures of chick embryonic cells, with subsequent testing of the oncogenicity of the cultures on chickens.

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